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SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM 1/

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FIELD TEST FOR THEBAINE ANALYSIS 2/

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1/ General note: The views expressed by collaborating scientists in their contribution to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Third Working Group on Papaver bracteatum (document ST/SOA/SER.J/15), it was recommended that a rough screening method should be developed for the determination of thebaine.

3/ This paper was submitted by Professor C.A. Salemink, Head of the Department of Organic Chemistry of Natural Products and Director of the Organic Chemical Laboratory, State University of Utrecht, The Netherlands.

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The following method for the detection of thebaine in fresh plant material might be very useful in the research on Papaver bracteatum in order to obtain a rapid and reliable indication of the presence of thebaine.

Minimum amount of fresh plant material

Leaf	:	2 g	(2 leaves of about 15 cm length)
Stem	:	1 g	(1 piece of stem, length 5 cm)
Root	:	1 g	(1 piece of root, average thickness: 0.5 cm, length: 10 cm)
Capsule	:	0.5 g	(1/3 - 1/2 part of one fresh capsule, length: 3-4 cm).

Materials and equipment

1. Merck SiO_2 "Fertigplatten" 15 x 1,5 cm.
2. Glass bottle, containing 250 ml of a saturated solution of concentrated ammonia (specific gravity 0.910) in diethylether.
3. Test tube (15 x 2 cm), containing 1 g of iodine crystals.
4. Test tube (15 x 2 cm), containing a strip of filter paper and 2 ml of "solvent system I" 5/ - toluene:acetone:methanol: ammonia 6 N (40:40:30:2).
5. Test tube (15 x 2 cm), containing a strip of filter paper and 2 ml of "solvent system II" 5/ - ethylacetate:methanol: ammonia 13 N (17:2:1).
6. Pasteur pipettes, watch glasses (diameter 10 cm), pair of scissors, mortar and pestle, test tube (12 x 1,1 cm), containing a solution of 50 mg thebaine in 10 ml diethylether.

4/ Suggestions for modifications which will improve the speed and reliability of the proposed method will be very welcome.

5/ G.C. Jain and M.S. Dahiya, Current Science (1974), 14, 444.

Procedure

1. Cut plant material into small pieces.

2. Macerate with 25 ml of diethylether/ammonia for 3 minutes.

3. Pipette the ether layer into a watch glass and, with gentle swirling, let the solvent evaporate to a small volume 6/.

4. Put two drops of the concentrated extract on a thin layer strip, as shown in Figure 1, in line with two reference spots of thebaine.

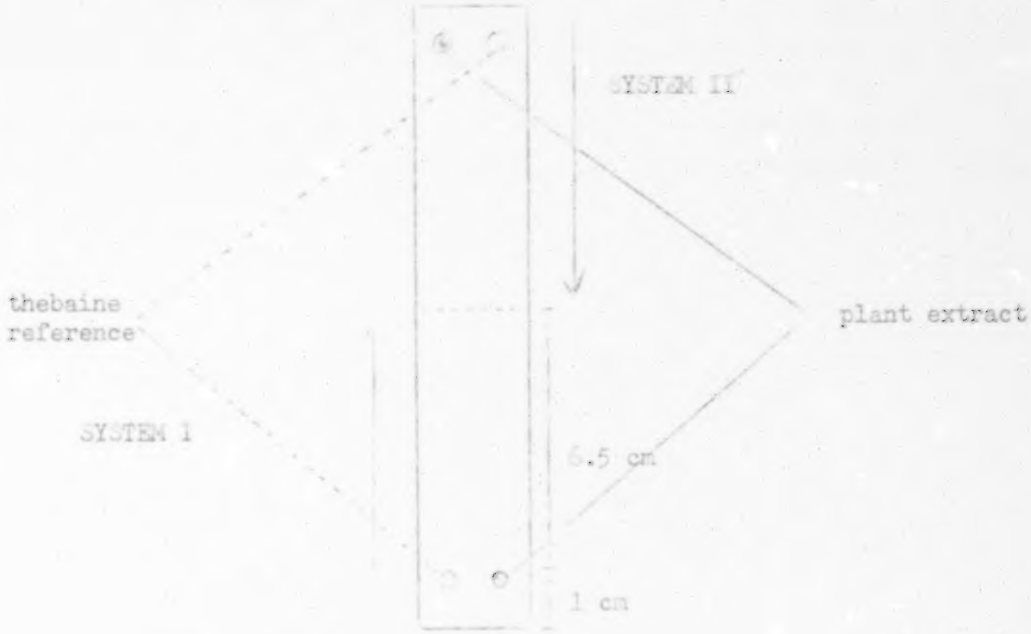

Figure 1

5. Place one of the "spotted sides" of the thin layer strip in solvent system 1. Let the solvent mixture elute until the solvent front reaches the dotted line (Figure 1). Next, remove the strip from the "system I tube" and dry in air for about 5 minutes.

6/ Note: When small droplets of water separate from the ethereal extract, on the watch glass, add some ether, since the application of water to the thin layer plate will severely disturb the smooth elution of the extract spots.

6. Repeat this procedure after turning the strip and use "solvent system II" for the second elution.

7. Visualize the spots in the tube containing the iodine crystals (limit of detection: approximately 1 - 2 micrograms of thebaine; time of visualization at least 3 minutes).

8. When spots from the plant extracts correspond with the thebaine reference spots (Rf 0.15 in system I and Rf 0.50 in system II) the test is considered to be positive.

9. The concentration of the thebaine in the reference spot should be approximately equal to that of the "sample-thebaine". If necessary, repeat the procedure with an "adjusted reference concentration".

The above method was applied to young leaves of P. bracteatum Arya I, grown in The Netherlands and reproducible results were obtained.